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The Inhibitory Effects of Hydrocortisone on Human Glioma Cells in Vitro

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Introduction

In view of the fact that a clinical report has been recently made that combined vasopressin-corticosteroid therapy is remarkably effective against recurrent malignant astrocytoma[1], it is considered to be necessary to review the anti-tumor action of corticosteroids. Due to the lack of precise methods to evaluate the effects of corticosteroids on the growth of human brain tumors, the differential response, if any, of individual tumors to such drugs has not been adequately studied. With the aim of studying whether corticosteroids have inhibitory action against human glioma or not, an attempt was made to evaluate this action in vitro with the expectation that it would reflect to some extent the action in vivo. In the present experiments the effects of hydrocortisone on the proliferation of cultured tumor cells derived from human astrocytoma-glioblastoma groups were studied.

Materials and Methods

Seventeen cases of astrocytoma-glioblastomas employed in the present experiments were classified histologically into 8 cases of glioblastoma (grade Ⅲ and Ⅳ) and 9 cases of astrocytoma (grade Ⅰ and Ⅱ). Except for Case 7 in which the tumor developed in the brain stem (in this case the cerebrospinal fluid tumor cells[5] were employed and the tumor tissue was confirmd later by autopsy) and Case 8 in which the tumor was found in the fourth ventricle, tumor developed in the cerebral hemisphere in the remaining 15 cases. The tumor tissue finely cut into small pieces was incubated in bottles and the cells migrating from the tissue fragments were subjected to the monolayer culture[9]. A procedure for trapping migrating cells from the fragments into a net of glass fibers and for subseqent cultivation of the trapped cells as a monolayer has also been successfully developed[4]. The cells were grown and maintained in the basic cultivating medium HAM-F 12 (Nissan Ltd.) supplemented with 20% fetal calf serum.

From the results of preliminary experiments using Ehrlich and HeLa cells, 1×10^{-4} g/ml of hydrocortisone sodium succinate (Solu-Cortef) in the medium was chosen as the highest concentration and from this concentration three serial dilutions of 10^{-5},

10^{-6} and 10^{-7} were prepared. When a sufficient number of cells were available in several cases, the effect of methylprednisolone sodium succinate (Solu-Medrol) was also tested. About 3 ml of cell suspension medium having a count of 2×10^4/ml was inoculated in 6 Leighton tubes, in duplicated numbers, containing coverslips. After confirming monolayer culture formation on the following day, the controls as of the commencement of the experiment were removed and the remaining tubes were replaced by growth medium containing 0, 10^{-4}, 10^{-6} and 10^{-7} of the test agents. Each tube was sealed with a rubber stopper and incubated at 37°C continuously for 7 days with the medium exchanged every two days. The cell count at the beginning and end of the experiment was determined after trypsinization by use of a hemacytometer and in some cases after Giemsa staining with the coverslips by counting the number of cell nuclei within a given field.

Results

The rate of cellular proliferation differs not only by oncotype but also by age of cells in vitro even with material taken from the same case and is not correlated with the degree of histological malignancy. Table 1 shows the results obtained on the 17 cases studied in this experiment. The proliferation rate indicates the calculated proliferation fold as 1.0 at the beginning of the experiment. Also are shown in percent in the parenthesis the rate of cell proliferation according to the concentration of the test agent using 100% as the untreated control. Two or three experiments were conducted for each histological type by different periods of culture and the mean rates obtained are shown in Figure 1. The overall relationship of hydrocortisone

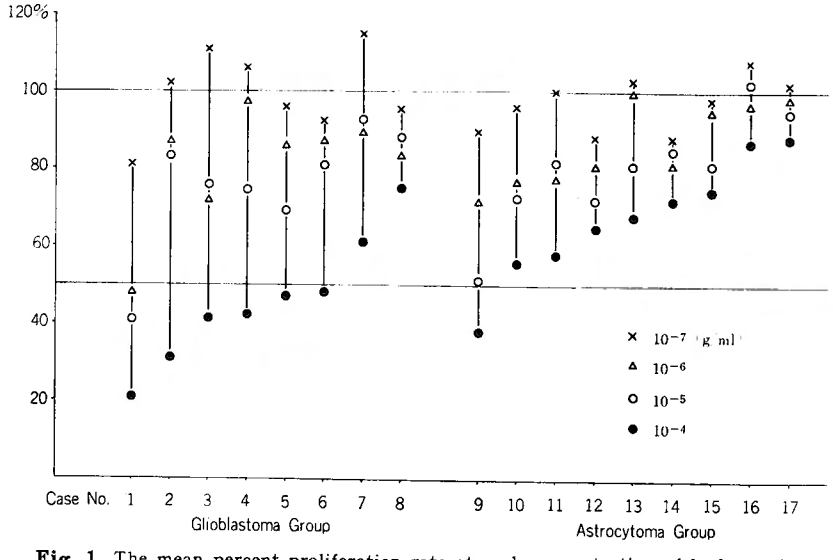

Fig. 1 The mean percent proliferation rate at each concentration of hydrocortisone (Untreated=100)

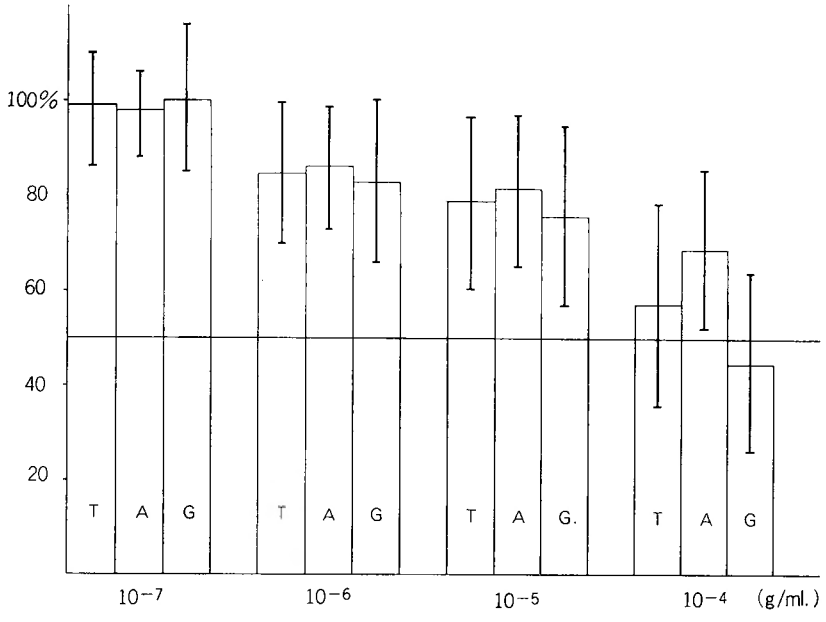

Fig. 2 The inhibitory effects of hydrocortisone according to histological types. : (T) Total, 17 cases, 40 experiments, (A) Astrocytoma, 9 cases, 20 experiments, (G) Glioblastoma, 8 cases, 20 experiments. (Untreated=100).

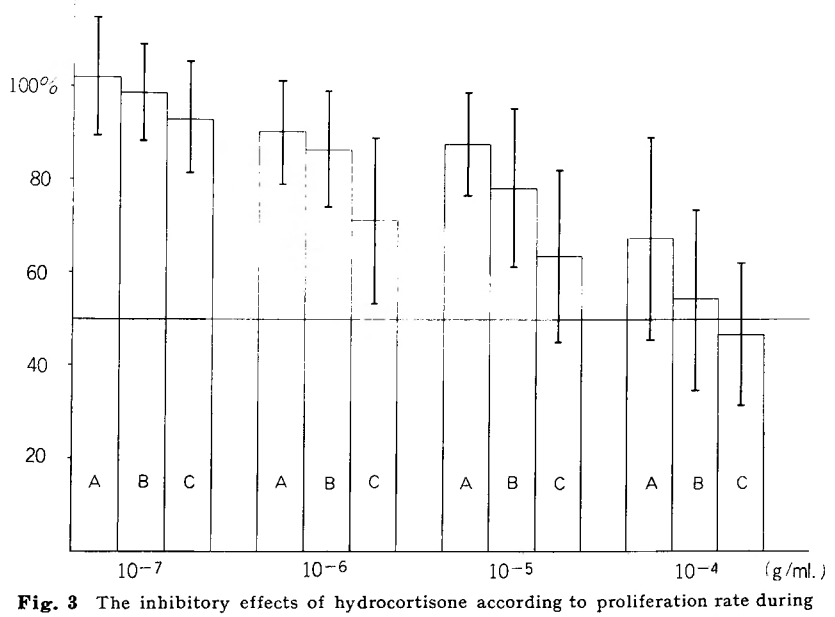

Fig. 3 The inhibitory effects of hydrocortisone according to proliferation rate during 7 days culture. : (A) less than 2.5,10 astro. and 6 gliobl., (B) 2.5-5.0,6 astro. and 9 gliobl., (C) more than5.0,4 astro. and 5 gliobl. (Untreated=100).

Table 1. The inhibitory effects of hydrocortisone on cellular proliferation during 7 days culture. (No.1—8; glioblastoma cases, No.9—17 ; astrocytoma cases)

No. Age, Sex, Histological type, Grade	Age of cells (days)	Proliferation fold (start ; 1.0) (percent value, untreated ; 100)				
		0	10^{-7}	10^{-6}	10^{-5}	10^{-4} (g/ml.)
1. 24,F Astrocytoma (Ⅳ)	18	7.2	5.4	2.7	1.7	1.6
		(100)	(75)	(38)	(23)	(22)
	36	6.4	5.6	3.6	3.8	1.3
		(100)	(88)	(57)	(60)	(21)
2. 37,F Astrocytoma (Ⅳ)	15	4.0	4.4	3.3	2.6	1.1
		(100)	(110)	(82)	(66)	(27)
	40	4.3	4.0	4.0	3.9	1.5
		(100)	(92)	(93)	(91)	(36)
	72	3.6	3.7	3.2	3.3	1.1
		(100)	(104)	(88)	(93)	(30)
3. 56,M Astrocytoma (Ⅲ)	16	5.2	5.4	3.0	3.3	3.0
		(100)	(103)	(57)	(63)	(58)
	50	2.4	2.9	2.0	2.1	0.6
		(100)	(120)	(85)	(88)	(24)
4. 63,M Astrocytoma (Ⅲ)	26	2.2	2.2	2.3	1.7	0.7
		(100)	(102)	(104)	(75)	(30)
	35	4.5	4.8	3.8	3.3	2.8
		(100)	(106)	(85)	(73)	(63)
	105	2.4	2.7	2.5	1.8	0.8
		(100)	(112)	(105)	(75)	(35)
5. 37,F Astrocytoma (Ⅲ)	35	4.6	5.1	4.6	1.9	1.7
		(100)	(111)	(100)	(41)	(36)
	46	7.0	5.6	5.5	5.9	4.6
		(100)	(80)	(78)	(84)	(65)
	62	3.0	2.9	2.4	2.5	1.2
		(100)	(96)	(81)	(84)	(41)
6. 24,F Astrocytoma (Ⅲ)	20	3.0	2.8	2.6	2.4	1.2
		(100)	(92)	(85)	(79)	(40)
	64	2.8	2.1	2.1	1.9	1.3
		(100)	(75)	(76)	(68)	(45)
	117	2.3	2.5	2.3	2.2	1.4
		(100)	(108)	(101)	(95)	(60)
7. 42,F Astrocytoma (Ⅲ)	24	5.9	6.8	5.5	5.3	2.4
		(100)	(155)	(94)	(90)	(40)
	55	2.1	2.5	1.8	2.0	1.7
		(100)	(117)	(88)	(95)	(82)
8, 13,F Asrtrocytoma (Ⅲ)	17	4.3	5.0	4.4	4.5	3.8
		(100)	(117)	(102)	(105)	(88)
	28	2.3	1.7	1.5	1.6	1.4
		(100)	(75)	(67)	(71)	(62)

9,	44,M Astrocytoma (II)	18	6.0	5.2	5.4	2.9	2.7
			(100)	(86)	(90)	(49)	(45)
		37	4.3	4.0	2.4	2.3	1.4
			(100)	(92)	(55)	(53)	(33)
10.	44,F Astrocytoma (II)	41	2.3	1.9	1.8	1.7	1.3
			(100)	(83)	(77)	(74)	(55)
		49	1.3	1.4	1.0	1.0	0.8
			(100)	(110)	(80)	(77)	(60)
11.	54,F Astro—Oligo. (I)	19	5.7	6.0	3.4	3.5	3.0
			(100)	(105)	(59)	(61)	(52)
		32	5.0	4.8	4.9	5.2	3.3
			(100)	(95)	(98)	(103)	(66)
12.	6,M Astrocytoma (II)	21	6.0	5.2	5.4	2.9	2.7
			(100)	(86)	(90)	(49)	(45)
		36	5.8	5.6	5.0	4.5	3.4
			(100)	(96)	(87)	(78)	(59)
		55	4.4	4.4	3.5	3.3	3.4
			(100)	(101)	(79)	(74)	(78)
13.	42,M Astrocytoma (II)	25	3.9	3.7	4.1	2.7	1.9
			(100)	(94)	(106)	(68)	(57)
		61	3.7	4.1	3.4	3.5	3.0
			(100)	(110)	(93)	(95)	(80)
14.	13,M Oligo-Astro. (I)	24	2.7	2.3	2.1	2.2	1.9
			(100)	(87)	(77)	(81)	(70)
		39	2.5	2.2	2.2	2.3	2.0
			(100)	(86)	(87)	(90)	(78)
15.	18,M Astrocytoma (I)	20	1.5	1.5	1.3	1.2	1.2
			(100)	(103)	(85)	(82)	(81)
		53	1.8	1.7	1.4	1.5	1.2
			(100)	(92)	(78)	(82)	(69)
16.	10,M Astrocytoma (I)	14	1.2	1.4	1.2	1.3	1.1
			(100)	(116)	(104)	(108)	(95)
		27	1.7	1.9	1.7	1.8	1.6
			(100)	(111)	(100)	(105)	(97)
		54	1.3	1.3	1.2	1.3	0.9
			(100)	(97)	(91)	(100)	(73)
17.	52,F Astrocytoma (I)	17	1.6	1.7	1.6	1.6	1.5
			(100)	(108)	(97)	(100)	(93)
		28	1.4	1.3	1.3	1.3	1.2
			(100)	(93)	(96)	(92)	(85)

effect on suppression of cellular proliferation according to histological malignancy is shown in Figure 2.

As shown in Figure 3, at the concentration of 10^{-7}, corresponding to the physiological level in human plasma, hardly any effect could be observed. But at 10^{-6} and 10^{-5} the mean proliferation was suppressed to about 80% and at 10^{-4} to about 60%. And in overall no significant difference in effect was observed at 10^{-7}, 10^{-6} and 10^{-5} according to histological type, but at the concentration of 10^{-4} a significant difference was apparently observed with the greater suppression on the malignant types. Moreover, as shown in Figure 1, at this highest concentration 6 out of 8 glioblastoma cases, but only one out of 9 astrocytoma cases, demonstrated a suppression rate exceeding 50%. In addition, though the suppressive effect differs according to the individual experiments as shown in Table 1, the overall suppressive effect was found to be greater on the cells having higher proliferative activity as shown in Figure 3.

Discussion

The therapeutic effects of corticosteroids on patients with brain tumor can be best explained by the decrease in cerebral edema and by the suppressed production rate of cerebrospinal fluid. Also supporting this hypothesis are the observations that these drugs relieve symptoms attributable to cerebral edema in patients with craniotomy, head injury, and other non-tumorous disorders of the brain. It has been clinically observed that corticosteroid therapy rapidly improves the impairment of consciousness or symptoms caused by brain edema or intracranial hypertension.

On the other hand, the administration of corticosteroid alone to glioma patients, even with imcompletely resected, sometimes brings about palliation for an extended period. We have not infrequently encountered patients in whom the anti-tumor action of corticosteroid was strongly suggested, though not compelling from the standpoint of its action of lowering intracranial pressure. As mentioned earlier, BERNARD-WEIL et al. have recommended as a new non-surgical treatment for glioma the so-called combined vasopressin-corticosteroid therapy by demonstrating its remarkable effects in clinical cases[1]. However, it may be said that in the practical application of this therapy there are a number of intuitive phases requiring further experimental studies.

A good many studies have been made on the anti-edema action of corticosteroid on the brain tissue around the tumor, its anti-inflammation action and its anti-cerebrospinal fluid production action. Even in this therapy there is no doubt that they are especially important factors during the early period of this therapy. It should, however, be evaluated from these authors' cases that in the long term administration their effects on the growth of tumor per se are also important factors. It is necessary to study what one can specifically expect from the so-called anti-tumor action of corticosteroid and what action mechanism is responsible for this.

Very little information is presently available on its relationship to tumors of the

central nervous system. According to the reports published in the past, it was BRZUSTOWICZ et al[2]. who observed the inhibitory effect of cortisone on the growth of subcutaneously transplanted ependymoma of the myxopapillary type in C_3H mice. According to KOTSILIMBAS et al.,[6] the administration of corticosteroid on mice with intracerebral melanomata brought about inhibition of symptoms, prolongation of life and suppression of tumor growth. WRIGHT et at.[10] have reported that the growth of astrocytoma-oligodendroglioma subcutaneously transplanted on rats was arrested by the administration of methylprednisolone begun after transplantation and that the growth of epedymoma subcutaneously transplanted on mice was remarkably inhibited by the administration of this steroid. GURCAY et al[3]. in studying the effect of methylprednisolone on malignant glioma transplanted in the brain of rats were also to demonstrate the anti-tumor action of corticosteroid. According to SHAPIRO et al.,[8] maximum increased survival of 24% over nontreated animals required a dose of 40 mg/kg of dexamethasone daily beginning the day following tumor implantation. The above mentioned data suggest that corticosteroid has, in addition to anti-edema action, a definite anti-tumor action, but there are still a number of unresolved problems, such as type of tumor, site of transplantion, dose and period of corticoid administration, host immunity etc.

In the present experiment the effect of hydrocortisone on proliferation of cells derived from astrocytoma-glioblastoma group was studied. Because of a lack of sufficient numbers of experiments using more potential methylprednisolone the results are not described in the present report. But it was found that these glucocorticoids inhibited cellular proliferation of gliomas. However, though the cells used in this experiment are empirically and morphologically considered to be tumor cells, there is no exact assurance that the neoplastic cells are being cultivated rather than stromal elements of the tumors. In this respect it seems to be important that no cells are being cultivated from normal brain tissue accrding to present culture method. Furthermore there are other disadvantages accompanying in vitro experiments, e. g. morphological and biological changes of cells etc. Regardless of these weakness the results of the present experiments using human materials can be still considered to be informative. MEALEY et al.[7] in 1971 also reported the effects of dexamethasone and methylprednisolone on cell cultures of 8 human glioblastomas. They and also I find a growth-inhibiting effect at high non-physiological levels of corticosteroids, at levels that might well be inhibitory to all cells normal or malignant, whether glioma or not. The present results show that the cellular proliferation is suppressed at doses of 100 micrograms per ml., in spite of the individual difference of response to steroid according to cell types. This represents the sum of the data of the paper. Although it goes without saying that the in vitro results should not be directly applied to those in vivo, if these data are to have any meaning for rational tumor therapy, one must see control tissue and consider that the patient would have to receive daily

massive doses of steroid to achieve extraordinary high concentrations of 100 micrograms/ml. in extracellular fluid. In this respect I agree with MEALEY et al. who emphasized the need to investigate additional, possibly more efficacious steroid compounds. But I belive from my clinical experience that it should be possible to effectively include steroid therapy in the program of treatment for glioma patients in whom recurrence is generally almost inevitable.

Summary

As a basic study on the vasopressin-corticosteroid therapy recommended by Bernard-Weil et al. for the treatment of glioma especially recurrent glioblastoma, a study was made on the effect of hydrocortisone on proliferation of cells in vitro obtained from 9 cases of astrocytoma and 8 cases of glioblastoma. Actively growing monolayer culture of tumor cells was exposed to the test agent of serially diluted concentration from 10^{-4} to 10^{-7} g/ml. in the medium.

The effectiveness was estimated by calculating the proliferation fold for 7 days. Within the concentration range used, the higher the concentration of hydrocortisone, the greater was the suppression rate. However, definite suppressive effect was achieved at doses of 10^{-4} g/ml. The possible roles of steroid for rational tumor therapy from the standpoint of its anti-tumor action were discussed.

Acknowledgment

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和文抄録

培養脳腫瘍細胞（人グリオーマ由来）における Hydrocortisone による増殖抑制効果の検討

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梶川　博・原田　廉・児玉　求

Bernard-Weil らによって提唱されたいわゆる vasopressin-corticosteroid 併用療法は，再発 glioblastoma，転移性脳腫瘍に対する新しい非手術療法として注目されてきている．この療法は，生理的には互いに培抗作用を持つとされている vasopressin と corticosteroid を併用することによって，両者に対する生体の feed back 機構を利用して，脳腫瘍患者にみられる "hypervasopressinism" の状態を是正し，併せて，contricosteroid の持つ，抗浮腫作用，髄液産生抑制作用，あるいは抗腫瘍作用など，脳腫瘍の治療上好ましい効果を期待するという仮説のもとに Bernard-Weil らが試み注目すべき成果をあげている療法である．しかしながら，本療法の施行に際しては，多くの研究課題が残されており，本稿では，まず，corticosteroid の抗腫瘍作用（腫瘍発育阻止作用）に対する評価を試みるべく，人の glioma（astrocytoma-glioblastoma）由来の細胞の in vitro における増殖に及ぼす hydrocortisone の影響について検討した．